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(73) Proprietor: THE WISTAR INSTITUTE
Thirty-Sixth Street at Spruce
Philadelphia Pennsylvania 19104-4268(US)

(72) Inventor: Tsujimoto,Yoshihide
249 Greene Avenue
Landsdowne, PA 19050(US)
Inventor: Croce,Carlos M
2401 Pine Street
Philadelphia, PA 19103(US)

(74) Representative: Dean, John Paul et al
Withers & Rogers 4 Dyer's Buildings Holborn
London EC1N 2JT (GB)

Rank Xerox (UK) Business Services
(3.10/3.6/3.3.1)

Description

BACKGROUND OF THE INVENTION

Specific chromosomal rearrangements, predominantly translocations and inversions, are observed in the great majority of human hematopoietic malignancies. In Burkitt lymphoma the specific chromosomal translocations result in the juxtaposition of one of the three human immunoglobulin loci and the c-myc oncogene. In diffuse B-cell lymphomas, multiple myelomas and chronic lymphocytic leukemias of the B-cell type carrying the t(11;14) (q13;q32) chromosome translocation, the bcl-1 locus is translocated to the heavy-chain locus on chromosome 14. In most cases of follicular lymphoma, one of the most common human hematopoietic malignancies, a (t14;18) (q32;q21) chromosome translocation has been observed. This translocation moves the bcl-2 gene to a position adjacent to the heavy-chain locus. In one cell line derived from a leukemic patient having both a t(14;8) and a t(14;18) translocation enhanced mRNA production from the bcl-2 gene was observed. (Tsujimoto et al, Science, Vol. 228, pages 1440-1443 (1985).) It was concluded there that the transcription unit of the bcl-2 gene spans the chromosome break-point, and thus the oncogene protein is likely to be structurally altered in the B-cell neoplasms. Surprisingly, it has now been found that the trans-location does not alter the oncogene protein itself, as the trans-location break-points occur downstream from the actual protein coding sequences. Thus oncogenesis may be solely due to the overproduction of the normal human gene products of the bcl-2 gene.

Effective treatment for cancer is often dependent upon an early and proper diagnosis of the malignancy. There is thus a need for simple and accurate diagnostic methods for detecting and identifying human malignancies, such as follicular lymphomas, in general, and follicular lymphomas in particular.

SUMMARY OF THE INVENTION

It is an object of the present Invention to provide a diagnostic method for detecting B-cell neoplasms having t(14;18) translocations in a human.

It is another object of the present invention to provide a human bcl-2 gene which can be expressed in bacteria.

It is yet another object of the present invention to provide a substantially pure protein preparation of a bcl-2 gene product.

It is still another object of the present invention to provide a diagnostic method of detecting B-cell neoplasms employing an antibody which is immunoreactive with a bcl-2 gene product.

These and other objects of the invention are achieved by one or more of the following embodiments.

In one embodiment a diagnostic method for detecting B-cell neoplasms in a human is provided, comprising the steps of:

isolating B-cells from the human;

extracting proteins from said B-cells to form a test sample;

contacting said test sample with an antibody which is immunoreactive with a gene product of the human bcl-2 gene, under conditions where antibody-antigen complexes are formed and are stable;

quantitating the amount of antibody-antigen complexes formed with said test sample; and

comparing the amount of antibody-antigen complexes formed with said test sample to the amount formed with a control sample of proteins, a ratio of test sample complexes to control sample complexes of greater than about ten indicating B-cell neoplasm, said control sample of proteins extracted from cells selected from the group consisting of: B-cells from a normal human, cells from an established normal B-cell or pre-B-cell line, and non-B-cells from said human.

Also provided are forms of the human bcl-2 gene which are substantially free of introns. Such genes can be replicated and expressed in bacteria to form proteins having the same primary structure as the bcl-2 proteins produced in humans.

Also provided by the present invention are substantially pure preparations of proteins having an N-terminal end encoded by the first exon of the human bcl-2 gene.

In yet another embodiment of the present invention a diagnostic method for detecting B-cell neoplasms in a human is provided comprising the steps of:

isolating B-cells from the humans;

extracting RNA from said B-cells to form a test sample;

contacting said test sample with a DNA probe containing a sequence of at least 15 nucleotides in length derived from the human bcl-2 gene, under conditions where homologous RNA-DNA hybrids form and are stable;

quantitating the amount of RNA-DNA hybrids formed with the test sample; and

comparing the amount of RNA-DNA hybrids formed with said test sample to the amount formed with a control sample of RNA, a ratio of test sample hybrids to control sample hybrids of greater than about ten indicating B-cell neoplasm, said control sample of RNA extracted from cells selected from the group consisting of: B-cells from a

normal human, cells from a normal B-cell or pre-B-cell line, and non-B-cells from said human.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a genomic restriction map of chromosome 18, and the structure of cDNA clones.

Figure 2 shows the nucleotide sequence of bcl-2 cDNA corresponding to the 5.5 kb transcript. Only the sequences surrounding the open reading frame are shown.

Figure 3 shows the nucleotide sequence of bcl-2 cDNA corresponding to the 3.5 kb transcript. Only the sequences surrounding the open reading frame are shown.

DETAILED DESCRIPTION OF THE INVENTION

According to one aspect of the present invention it has been discovered that B-cell neoplasms which are associated with t(14;18) chromosome translocations cause an increase in the expression of both the mRNA and the protein products of the bcl-2 gene. The expression in the neoplastic B-cells is generally about ten-fold higher than the amount expressed by normal cells. This elevated expression can be used as a diagnostic tool to detect the B-cell neoplasms in humans. Such neoplasms include follicular lymphomas as well as other lymphomas.

It has been found that three species of mRNA are transcribed from the bcl-2 gene. At least two different protein products have been identified. A 239 amino acid protein, designated bcl-2-alpha is translated from a 5.5 kb mRNA. A protein of 205 amino acids, bcl-2-beta is translated from a 3.5 kb mRNA. It is thought that bcl-2-alpha is also translated from the 8.5 kb mRNA species. All three size species of transcript share substantial sequence homology In the 5' portion of the gene, termed the first exon or 5' exon. The larger two transcripts appear to be spliced to a second exon which is at least 50 kb distal to the first. The splice site is in the midst of the protein coding sequence. Thus, the 3.5. kb transcript codes for a protein having a different carboxyl terminus than the proteins coded by the two larger sized transcripts.

The hotspot for chomosome break-points among the t(14;18) translocations of follicular lymphomas map 3' to the protein coding region. Therefore it has now been found that the translocations do not alter the primary structure of the protein products.

Bacterial isolates available from the ATCC, under Accession Numbers 67147 and 67148, can be used to express bcl-2 gene products alpha and beta, respectively, in bacteria. The clones of the bcl-2 gene were obtained via cDNA cloning and so do not contain introns. Thus these clones can be expressed in bacteria to make products having the same primary sequence as those made in the human body. After growing the bacteria under suitable conditions which are well known in the art, the cells can be harvested and disrupted to extract total cellular protein. The protein can then, for example, be placed on a sizing column such as Sepharose™ or agarose beads, and proteins of the correct molecular weight, i.e., between about 20 and 30 kD can be collected.

Further purification can be effected by use of an anti-bcl-2 antibody. Such an antibody can be used to immunoprecipitate bcl-2 proteins from the set of cellular proteins of the correct approximate molecular weight. Such antibodies can, for example, be raised against polypeptides synthesized according to the sequence shown in Figures 2 and 3. Alternatively, the antibodies can be raised against fusion proteins, which contain bcl-2 sequences as well as those of another protein. An example of an antibody raised to a fusion protein is discussed, infra. After immunoprecipitation, the bcl-2 proteins can be released from the antibodies to provide a substantially pure preparation of bcl-2 proteins.

If it is desired that bcl-2-alpha (approximately 26 kD) be separated from bcl-2-beta (approximately 22 kD) this separation can be achieved using either polyacrylamide gels or additional sizing or gel filtration columns. Of course, other separations are possible based on the inherent differences between the two proteins at their carboxyl termini. Techniques for using gel fitration columns and immunoprecipitation and antibody releasing are all well known in the art.

Any source of B-cells is suitable for use in the diagnostic test of this invention. B-cells can be isolated from the lymph nodes. Alternatively, the diagnostic test may employ a sample of peripheral blood obtained from an individual who is being screened for the presence of a B-cell neoplasm. Means for separating B-cells from the peripheral blood are well known in the art. For example, erythrocytes and granulocytes may be separated from the B-cells by centrifugation in a liquid having a density intermediate between the groups of cells to be separated.

Extraction of proteins from B-cells may be performed by any of the many means known in the art. For example, cells may be lysed by a detergent or by mechanical means. If desired, nucleic acids can be removed from the cell preparation by enzymatic digestion or by precipitation with agents such as streptomycin. Once again, such means are well known in the art.

Antibodies can be generated which are immunoreactive with the bcl-2 proteins by immuniza-

tion of animals with a fusion protein consisting of a portion of the beta-galactosidase protein of E.coli and a portion of the human bcl-2 proteins. Preferably, the bcl-2 portion will contain sequences which are common to both bcl-2-alpha and bcl-2-beta. If desired, such a fusion protein can be purified using the properties which it shares with beta-galactosidase. Anti-sera raised against such a fusion protein in rabbits have been found to be immunoreactive with both bcl-2-alpha and bcl-2-beta in vitro. Furthermore, using this anti-sera in immunofluorescent techiques it is possible to determine cellular location of bcl-2 proteins in cells which have been fixed.

Antibodies can also be produced by immunization of animals, such as mice, rabbits and the like, with bcl-2-alpha, bcl-2-beta, fragments of them, or both. Alternatively, monoclonal antibodies can be generated using immortalized cell lines to provide uniform and continual antibody sources. Techniques for generating such antibodies are well known in the art. Appropriate antibodies can be screened using the natural gene products of bcl-2 or the fusion protein discussed above. While it is preferred that the antibody used in the diagnostic method immunoreact with both bcl-2-alpha and beta, an antibody may successfully be used which immunoreacts with only one of them.

The extracted proteins from the B-cells may be contacted with the antibody under suitable conditions for antibody-antigen complex formation. Generally, such conditions are physiological conditions. The protein extract may be bound to a solid support such as a nitrocellulose filter or a microtiter plate.

The antibody will generally bear a "tag" such as a radiolabel, a fluorescent label or an enzyme conjugate which under appropriate conditions produces a colored reaction product. Once again, such "tags" are quite well known in the art. Alternatively, if the antibody is not tagged, it can be detected by means of a second antibody from another species which is reacted with the first antibody. Of course, it is preferred for means of this diagnostic method that the immunological technique be as quantitatively sensitive as possible.

Means of detection of the antibody-antigen complexes will depend upon the method of tagging used for the antibody. For example, radiolabel tags can be detected by autoradiography or scintillation counting, while the products of enzyme-linked antibodies can be detected spectrophotometrically.

A parallel sample to the test sample is employed to provide the control. The control sample consists of an equivalent amount of proteins extracted from cells, preferably in the same manner as those of the test sample. The amount of protein can readily be determined employing techniques well known in the art, such as the Lowry or Bradford techniques. The cells used for preparing the control sample may be selected from the group consisting of B-cells from a normal human, cells from an established normal B-cell or pre-B-cell line, and non-B-cells from the human who is being screened for the neoplasm.

It is a finding of the present invention that in cases where a translocation has occurred between chromosomes 14 and 18 the level of bcl-2 protein detected immunologically in the B-cells is at least 10-fold higher than the amount of bcl-2 protein found in normal B-cells, in pre-B-cells, or in other non-B-cells from the same human.

To screen for elevated levels of mRNA transcribed from the bcl-2 gene, again one must isolate B-cells from the human who is to be screened. Any of the many methods known in the art are suitable. Total RNA extracted from the B-cells may be used, or alternatively mRNA may be isolated from the total cellular RNA. The mRNA may be purified, for example, by affinity chromotography on oligo(dT) cellulose which binds to the poly(A) tract at the 3' end of most mRNA. As is well known to those of skill in the art, it is essential that ribonuclease activity be minimized during preparation and assaying.

A DNA probe may be selected from any of the protein coding sequences of the bcl-2 gene. Preferably, the probe will be selected from sequences of the 5' or first exon of the gene, so that all three species of RNA can be detected. Alternatively the probe can be selected from sequences which hybridize exclusively with the 3.5 kb transcript or only with the 5.5 kb and the 8.5 kb transcript. Preferably the probe contains at least 15 nucleotides of the bcl-2 sequence. Suitable plasmid molecules which may be used as probes have been deposited at the ATCC under Deposit Numbers 67147 and 67148. Of course, other suitable probes may be synthesized or derived from these or other bcl-2 sequences. In order to perform the hybridization it is desirable that the probe be single stranded. Thus if the probe is double stranded, it should be denatured to single stranded form. Means for denaturing are well known in the art, including alkali or heat treatment. The probe can then be contacted with the RNA derived from the B-cells under conditions where homolgous RNA-DNA hybrids form and are stable. Such conditons are well known in the art. Means for detecting hybrids are many and well known, but often involves use of radiolabeled probes and nucleases which degrade single stranded DNA. Other methods may be used.

Control samples can be derived from any of the cell sources described above for use in the antibody diagnostic test. Samples and control should be prepared in parallel under similar con-

ditions. If comparison of the test and control sample hybridization shows a greater than about a tenfold excess in the test sample, a B-cell neoplasm is indicated.

The following examples do not limit the scope of the invention but are merely illustrative.

EXAMPLE 1

A cDNA library from polyA$^+$ mRNA of the pre-B-cell leukemia cell line 380 was constructed. Cytoplasmic RNA was extracted by the procedure described in ar-Rushdi, et al (1982) Somatic Cell Genetics, Vol. 8, pp. 151-161. PolyA$^+$ RNA was selected by oligo(dT) column chromotography as described in Aviv and Leder, (1972) Proceedings of National Academy of Sciences, USA, Vol. 69, pp. 1408-1412. Double stranded cDNA was synthesized from mRNA by reverse transcriptase (Life Science, Inc., Florida) using oligo(dT) as primer as described in Maniatis et al (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. After EcoRI linker ligation, the double stranded cDNA was cloned into lambda gt 11 phage vectors, Young and Davis (1983) Proceedings of the National Academy of Sciences, USA, Vol. 80, pp. 1194-1198. By screening approximately 2 x 10^5 recombinant clones with a DNA probe consisting of a segment of chromosome 18 which spans the hotspot of break-points of the translocation of chromosome 18 to chromosome 14, three independent cDNA clones were obtained which are overlapping (B3, B4, and B10). As shown in Figure 1, clone B3 contains nineteen A residues at the end, indicating that this clone represents the 3' end of the mRNA. The restriction maps of the cDNA clones and genomic sequences are colinear from the 3' end of cDNA clone B3 until just before the BamHI site of the cDNA sequence. The cDNA sequence just beyond this point diverges from the genomic sequence. Thus, the cDNA sequences consist of at least two genomic regions.

When the 5' part of cDNA clone B4 (5' end to the BamHI site) is used to probe the cDNA bank, another set of clones is obtained, including clone B15 and clone B16 (see Fig. 1). These two cDNA clones have the same sequences at the 5' region as does clone B4, but they have totally different sequences at the 3' region than do clones B3, B4, and B10. Thus cDNA cloning has yielded two different sets of clones, indicating that the bcl-2 gene is transcribed into at least two different mRNAs.

In order to obtain cDNA sequences further upstream (in the 5' direction), a cDNA library was constructed using the primer extension method. An oligonucleotide (15-mer) was synthesized and used as a primer for reverse transcriptase as described in Maniatis, supra, and in Gubler and Hoffman (1983) Gene, Vol. 25, pp. 263-269. Three clones were obtained by this method, clone B6-3, B22-1, and B9.

EXAMPLE II

Two different probes were used to visualize the mRNA species corresponding to the bcl-2 gene in Northern blot hybridizations. The first probe (probe A in Figure 1) contains genomic DNA of chromosome 18 which spans the break-point hotspot and also corresponds to the 3' exon. The other probe used was cDNA clone B22-1 which corresponds to the 5' exon (the first exon). RNA was glyoxalated, run on 1% agrose gels, and blotted to nitrocellulose filters as described in Thomas, Proceedings of the National Academy of Sciences, USA (1980) Vol. 77, pp. 5201-5205. The nitrocellulose filter was hybridized with ^{32}P-labelled probe in 50% formamide, 4 X SSC, 0.1% SDS at 37°C and finally washed with 2 X SSC (0.3M NaCl, 0.03 M Na-citrate, pH 7) at 65°C.

The genomic DNA probe A detected two transcripts, 8.5 kb and 5.5 kb in length. The cDNA probe B22-1 detected the same transcripts as probe A, as well as an additional transcript of 3.5 kb.

The 8.5 kb mRNA was also shown to hybridize to a gemonic DNA probe from chromosome 18 which is 3' to genomic probe A (and is indicated in Fig. 1 as probe B).

These data indicate that the bcl-2 gene is transcribed into three mRNAs of different sizes. The possibility that these mRNAs are derived from different but related genes is excluded by the fact that under the same hybridizatoin conditions which were used for the Northern blot hybridization, these probes detect only one cellular gene.

EXAMPLE III

The nucleotide sequence of overlapping cDNA clones was determined by Maxam and Gilbert's chemical degradation method, (Proceedings of the National Academy of Sciences, USA, Vol. 74, pp. 560-564 (1977), or Sanger's chain termination method, Proceedings of the National Academy of Sciences, USA, Vol. 74, pp. 5463-5467 (1977). Both strands of DNA were sequenced. The nucleotide sequence derived from the 5.5 kb transcript is shown in Figure 2. The DNA sequence of 5105 base pairs (bp) reveals one possible open reading frame consisting of 239 amino acid residues (bcl-2-alpha). The nucleotide sequence corresponding to the 3.5 kb transcript is shown in Figure 3. This transcript codes for a protein consisting of 205 amino acid residues (bcl-2-beta),

which differs from the bcl-2-alpha protein at the carboxyl terminus.

Claims

1. A diagnostic method for detecting B-cell neoplasms having t(14;18) translocations in a human, comprising the steps of: isolating B-cells from the human; extracting proteins from said B-cells to form a test sample; contacting said test sample with an antibody which is immunoreactive with a gene product of the human bcl-2 gene under conditions where antibody-antigen complexes form and are stable to form antibody-antigen complexes; quantitating the amount of antibody-antigen complexes formed with said test sample; comparing the amount of antibody-antigen complexes formed with said test sample to the amount formed with a control sample of proteins, a ratio of test sample complexes to control sample complexes of greater than about ten indicating B-cell neoplasm, said control sample of proteins extracted from cells selected from the group consisting of: B-cells from a normal human, cells from an established normal B-cell or pre-B-cell line, and non-B-cells from said human.

2. The method of claim 1 wherein the antibody is radiolabeled and the step of quantitating occurs by measuring the amount of radioactivity in antibody-antigen complexes.

3. The method of claim 1 wherein the antibody is conjugated to a fluorescent molecule and the step of quantitating is performed by measuring the amount of fluorescence emitted by said antibody-antigen complexes.

4. The method of claim 1 wherein said step of quantitating is performed by means of use of a second antibody immunoreactive with said antibody which is immunoreactive with a gene product of bcl-2 gene.

5. The method of claim 1 wherein the antibody is immunoreactive with bcl-2-alpha.

6. The method of claim 1 wherein the antibody is immunoreactive with bcl-2-beta.

7. The method of claim 1 wherein the antibody is immunoreactive with both bcl-2-alpha and bcl-2-beta.

8. A human bcl-2 gene substantially free of introns.

9. The gene of claim 8 wherein said gene is replicated in bacteria.

10. The gene of claim 9 wherein said gene is expressed in bacteria

11. A substantially pure preparation of protein having an N-terminal end encoded by the first exon of the human bcl-2 gene.

12. The protein preparation of claim 11 wherein said protein is bcl-2-alpha having 239 amino acid residues.

13. The protein preparation of claim 11 wherein said protein is bcl-2-beta having 205 amino acid residues.

14. A diagnostic method for detecting B-cell neoplasms having t(14;18) translocations in a human comprising the steps of: isolating B-cells from the human; extracting RNA from said B-cells to form a test sample; contacting said test sample with a DNA probe containing a sequence of at least 15 nucleotides in length derived from the human bcl-2 gene under conditions where homologous RNA-DNA hybrids form and are stable; quantitating the amount of RNA-DNA hybrids formed in the test sample; and comparing the amount of RNA-DNA hybrids formed with said test sample to the amount formed with a control sample of RNA, a ratio of test sample hybrids to control sample hybrids of greater than about ten indicating B-cell neoplasm, said control sample of RNA extracted from cells selected from the group consisting of: B-cells from a normal human, cells from a normal B-cell or pre-B-cell line, and non-B-cells from said human.

15. The method of claim 14 wherein said DNA probe hybridizes with the 8.5 kb, the 5.5 kb, and the 3.5 kb bcl-2 transcripts.

16. The method of claim 14 wherein said DNA probe hybridizes with the 8.5 kb and the 5.5 kb bcl-2 transcripts.

17. The method of claim 14 wherein said DNA probe hybridizes exclusively with the 3.5 kb bcl-2 transcript.

18. A preparation of an antibody which is (a) immunoreactive with a human bcl-2 protein selected from the group consisting of bcl-2-alpha and bcl-2-beta, and which is (b) not immunoreactive with other human proteins.

19. The preparation of claim 18 which is immunoreactive with both bcl-2-alpha and bcl-2-beta.

20. The preparation of claim 18 or 19 which has been purified from an animal antiserum.

21. The preparation of claim 18, 19 or 20 wherein the antibody is a monoclonal antibody.

Patentansprüche

1. Diagnostisches Verfahren zum Nachweis von t-(14;18)-Translokationen aufweisenden B-Zellen-Neoplasmen beim Menschen, das folgende Stufen umfaßt:
Isolierung der B-Zellen aus dem Menschen, Extraktion der Proteine aus den B-Zellen zur Bildung einer Testprobe, Kontaktierung der Testprobe mit einem Antikörper, der mit einem Genprodukt des menschlichen bcl-2-Gens unter Bedingungen immunreaktiv ist, bei denen Antikörper-Antigen-Komplexe gebildet werden und gegenüber der Bildung von Antikörper-Antigen-Komplexen beständig sind, Quantifizierung der Menge der mit der Testprobe gebildeten Antikörper-Antigen-Komplexe und Vergleich der Menge der mit der Testprobe gebildeten Antikörper-Antigen-Komplexe mit der mit einer Kontrollprobe von Proteinen gebildeten Menge, wobei ein Verhältnis der Testprobenkomplexe zu den Kontrollprobenkomplexen von über ca. 10 B-Zellen-Neoplasmen anzeigt und die Kontrollprobe von Proteinen extrahiert wird aus Zellen, ausgewählt aus der Gruppe, bestehend aus B-Zellen aus einem gesunden Menschen, Zellen aus einer festgelegten normalen B-Zell- oder prä-B-Zellinie und nicht-B-Zellen aus dem betreffenden Menschen.

2. Verfahren nach Anspruch 1, worin der Antikörper radioaktiv markiert wird und die Stufe der Quantifizierung durch Messung der Menge der Radioaktivität in den Antikörper-Antigen-Komplexen erfolgt.

3. Verfahren nach Anspruch 1, worin der Antikörper mit einem fluoreszierenden Molekül konjugiert wird und die Stufe der Quantifizierung durch Messung der Menge der von den Antikörper-Antigen-Komplexenabgegebenen Fluoreszenz erfolgt.

4. Verfahren nach Anspruch 1, worin die Stufe der Quantifizierung mit Hilfe eines zweiten Antikörpers durchgeführt wird, der gegenüber dem ersten Antikörper immunreaktiv ist, der mit einem Genprodukt des bcl-2-Gens immunreaktiv ist.

5. Verfahren nach Anspruch 1, worin der Antikörper gegenüber bcl-2-α immunreaktiv ist.

6. Verfahren nach Anspruch 1, worin der Antikörper gegenüber bcl-2-β immunreaktiv ist.

7. Verfahren nach Anspruch 1, worin der Antikörper sowohl gegenüber bcl-2-α als auch gegenüber bcl-2-β immunreaktiv ist.

8. Ein im wesentlichen intronfreies menschliches bcl-2-Gen.

9. Das Gen nach Anspruch 8, das in Bakterien repliziert wird.

10. Das Gen nach Anspruch 9, worin das Gen in Bakterien exprimiert wird.

11. Ein im wesentlichen reines Proteinpräparat mit einem N-Terminus, der durch das erste Exon des menschlichen bcl-2-Gens kodiert wird.

12. Proteinpräparat nach Anspruch 11, worin das Protein 239 Aminosäurereste aufweisendes bcl-2-α ist.

13. Proteinpräparat nach Anspruch 11, worin das Protein 205 Aminosäurereste aufweisendes bcl-2-β ist.

14. Diagnostisches Verfahren zum Nachweis von t-(14;18)-Translokationen aufweisenden B-Zellen-Neoplasmen beim Menschen, das folgende Stufen umfaßt:
Isolierung von B-Zellen aus dem Menschen,
Extraktion der RNA aus den B-Zellen zur Bildung einer Test-probe,
Kontaktierung der Testprobe mit einer DNA-Probe, die eine Sequenz von wenigstens 15 Nukleotiden in der Länge aufweist, die aus dem menschlichen bcl-2-Gen erhalten wurden, unter Bedingungen, bei denen homologe RNA-DNA-Hybride entstehen und beständig sind,
Quantifizierung der Menge an in der Testprobe gebildeten RNA-DNA-Hybriden und Vergleich der mit der Testprobe gebildeten Menge an RNA-DNA-Hybriden mit der mit einer Kontrollprobe von RNA gebildeten Menge, wobei ein Verhältnis der Testprobenhybriden zu den Kontrollprobenhybriden von über ca. 10 B-Zellen-Neoplasmen anzeigt und die Kontrollprobe von RNS extrahiert wird aus Zellen, ausgewählt aus der Gruppe, bestehend aus B-Zellen eines gesunden Menschen, Zellen aus einer normalen B-Zell- oder prä-B-Zellinie und

nicht-B-Zellen aus dem betreffenden Menschen.

15. Verfahren nach Anspruch 14, worin die DNA-Sonde mit dem 8,5 kb-, 5,5 kb- und 3,5 kb-bcl-2-Transkript hybridisiert.

16. Verfahren nach Anspruch 14, worin die DNA-Sonde mit dem 8,5 kb- und 5,5 kb-bcl-2-Transkript hybridisiert.

17. Verfahren nach Anspruch 14, worin die DNA-Sonde ausschließlich mit dem 3,5 kb-bcl-2-Transkript hybridisiert.

18. Präparat eines Antikörpers, der (a) gegenüber einem menschlichen bcl-2-Protein immunreaktiv ist, ausgewählt aus der Gruppe bcl-2-α und bcl-2-β, und (b) gegenüber anderen menschlichen Proteinen nicht immunreaktiv ist.

19. Präparat nach Anspruch 18, das sowohl gegenüber bcl-2-α als auch gegenüber bcl-2-β immunreaktiv ist.

20. Präparat nach Anspruch 18 oder 19, das durch Reinigung aus einem tierischen Antiserum gewonnen wurde.

21. Präparat nach Anspruch 18, 19 oder 20, worin der Antikörper ein monoklonaler Antikörper ist.

Revendications

1. Procédé diagnostique pour la détection de néoplasmes à cellules B ayant les translocations t(14;18) chez un homme, comprenant les étapes suivantes : isolation des cellules B de l'homme; extraction des protéines à partir de ces cellules B pour former un échantillon d'essai; mise en contact de cet échantillon d'essai avec un anticorps qui est immunoréactif avec un produit génique du gène bcl-2 humain dans des conditions dans lesquelles les complexes anticorps-antigène se forment et sont stables pour former des complexes anticorps-antigène; détermination de la quantité de complexes anticorps-antigène formés avec cet échantillon d'essai; comparaison de la quantité de complexes anticorps-antigène formés avec cet échantillon d'essai à la quantité formée avec un échantillon témoin de protéines, un rapport des complexes de l'échantillon d'essai aux complexes de l'échantillon témoin supérieur à environ 10 indiquant un néoplasme à cellules B, cet échantillon témoin de protéines extraites provenant de cellules sélectionnées dans le groupe consistant en cellules B provenant d'un homme normal, cellules provenant d'une lignée de cellules B normale établie ou de précellules B et cellules non B provenant de cet homme.

2. Procédé suivant la revendication 1, dans lequel l'anticorps est radiomarqué et l'étape de détermination quantitative est réalisée par mesure de la quantité de radioactivité dans les complexes anticorps-antigène.

3. Procédé suivant la revendication 1, dans lequel l'anticorps est conjugué à une molécule fluorescente et l'étape de détermination quantitative est réalisée par mesure de la quantité de fluorescence émise par ces complexes anticorps-antigène.

4. Procédé suivant la revendication 1, dans lequel cette étape de détermination quantitative est réalisée au moyen d'un second anticorps immunoréactif avec cet anticorps qui est immunoréactif avec un produit génique du gène bcl-2.

5. Procédé suivant la revendication 1, dans lequel l'anticorps est immunoréactif avec bcl-2-alpha.

6. Procédé suivant la revendication 1, dans lequel l'anticorps est immunoréactif avec bcl-2-béta.

7. Procédé suivant la revendication 1, dans lequel l'anticorps est immunoréactif à la fois avec bcl-2-alpha et bcl-2-béta.

8. Gène bcl-2 humain pratiquement dépourvu d'introns.

9. Gène suivant la revendication 8, dans lequel ce gène est répliqué dans des bactéries.

10. Gène suivant la revendication 9, dans lequel ce gène est exprimé dans des bactéries.

11. Préparation pratiquement pure de protéine ayant une extrémité N-terminale codée par le premier exon du gène bcl-2 humain.

12. Préparation de protéine suivant la revendication 11, dans laquelle cette protéine est bcl-2-alpha ayant 239 résidus d'acide aminé.

13. Préparation de protéine suivant la revendication 11, dans laquelle cette protéine est bcl-2-béta ayant 205 résidus d'acide aminé.

14. Procédé diagnostique pour la détection de néoplasmes à cellules B ayant les transloca-

tions t(14;18) chez un homme, comprenant les étapes suivantes : isolation des cellules B de l'homme; extraction de l'ARN de ces cellules B pour former un échantillon d'essai; mise en contact de cet échantillon d'essai avec une sonde d'ADN contenant une séquence longue d'au moins 15 nucléotides provenant du gène bcl-2 humain dans des conditions dans lesquelles des hybrides homologues ARN-ADN se forment et sont stables; détermination de la quantité d'hybrides ARN-ADN formés dans l'échantillon d'essai; et comparaison de la quantité d'hybrides ARN-ADN formés avec cet échantillon d'essai à la quantité formée avec un échantillon témoin d'ARN, un rapport d'hybrides dans l'échantillon d'essau aux hybrides dans l'échantillon témoin supérieur à environ 10 indiquant un néoplasme à cellules B, cet échantillon témoin d'ARN étant extrait à partir de cellules sélectionnées dans le groupe consistant en cellules B provenant d'un homme normal, cellules provenant d'une lignée de cellules B normale ou de pré-cellules B et cellules non B provenant de cet homme.

15. Procédé suivant la revendication 14, dans lequel cette sonde d'ADN s'hybride avec les transcripts de 8,5 kb, de 5,5 kb et de 3,5 kb de bcl-2.

16. Procédé suivant la revendication 14, dans lequel cette sonde d'ADN s'hybride avec les transcripts de 8,5 kb et de 5,5 kb de bcl-2.

17. Procédé suivant la revendication 14, dans lequel cette sonde d'ADN s'hybride exclusivement avec le transcript de 3,5 kb de bcl-2.

18. Préparation d'un anticorps, qui est (a) immunoréactive avec une protéine bcl-2 humaine choisie dans le groupe consistant en bcl-2-alpha et bcl-2-béta, et qui est (b) non immunoréactive avec d'autres protéines humaines.

19. Préparation suivant la revendication 18, qui est immunoréactive à fois avec bcl-2-alpha et bcl-2-béta.

20. Préparation suivant les revendications 18 ou 19, qui a été purifiée à partir d'un antisérum humain.

21. Préparation suivant les revendications 18, 19 ou 20, dans laquelle l'anticorps est un anticorps monoclonal.

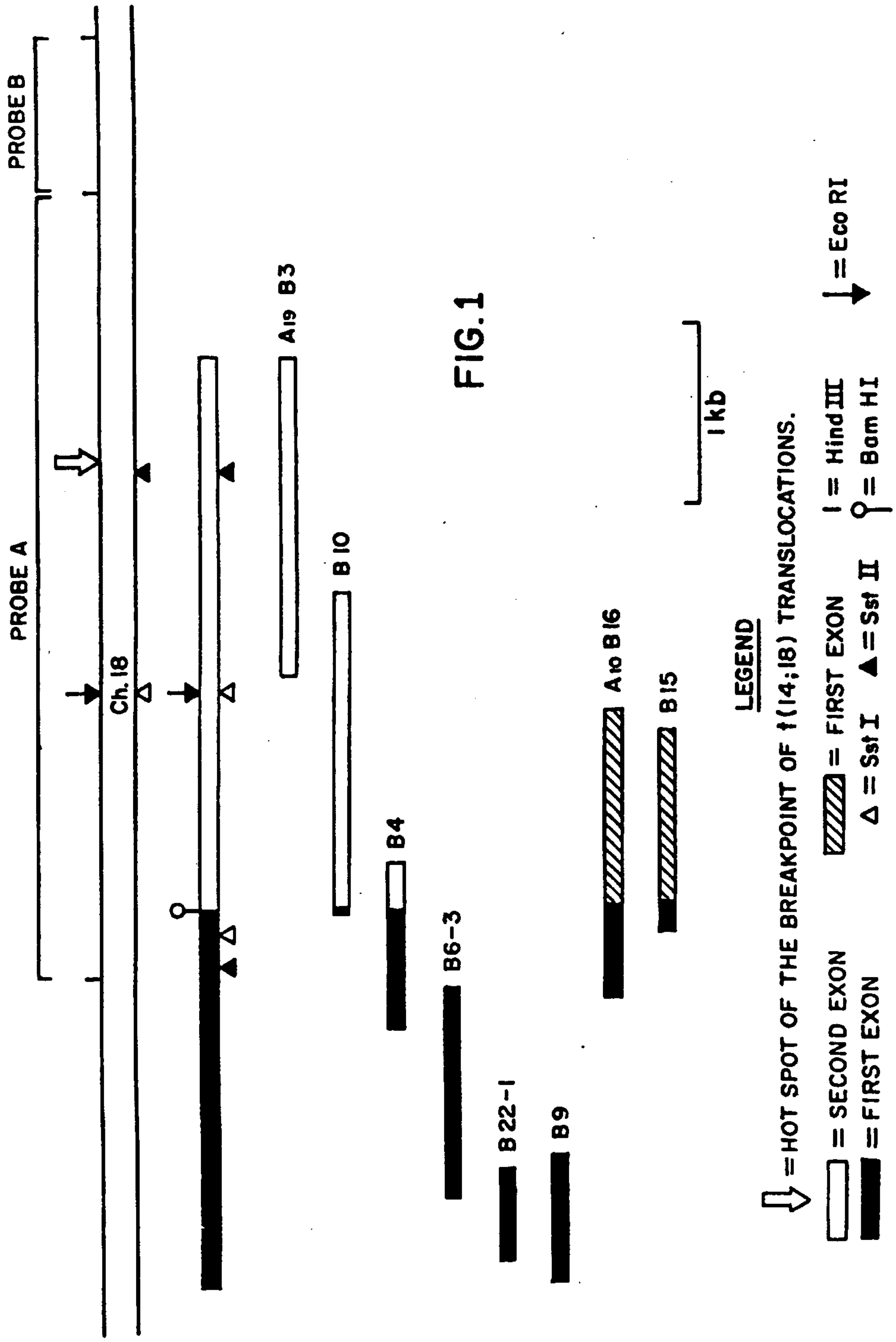
PROBE B
PROBE A
Ch. 18
A19 B3
B10
B4
B6-3
B22-1
B9
A10 B16
B15
1 kb
LEGEND
= HOT SPOT OF THE BREAKPOINT OF t(14;18) TRANSLOCATIONS.
= SECOND EXON
= FIRST EXON
= FIRST EXON
Δ = Sst I
▲ = Sst II
I = Hind III
= Bam HI
= Eco RI

FIG. 1

```
                                                    GCGCCCGC
CCCTCCGCGC CGCCTGCCCG CCCGCCCGCC GCGCTCCCGC CCGCCGCTCT    -1401
CCGTGGCCCC GCCGCGCTGC CGCCGCCGCC GCTGCCAGCG AAGGTGCCGG
GGCTCCGGGC CCTCCCTGCC GGCGGCCGTC AGCGCTCGGA GCGAACTGCG
CGACGGGAGG TCCGGGAGGC GACCGTAGTC GCGCCCGCCGC GCAGGACCAG
GAGGAGGAGA AAGGGTGCGC AGCCCGGAGG CGGGGTGCGC CGGTGGGGTG
CAGCGGAAGA GGGGGTCCAG GGGGGAGAAC TTCGTAGCAG TCATCCTTTT
TAGGAAAAGA GGGAAAAAAT AAAACCCTCC CCCACCACCT CCTTCTCCCC
ACCCCTCGCC GCACCACACA CAGCGCGGGC TTCTAGCGCT CGGCACCGGC
GGGCCAGGCG CGTCCTGCCT TCATTTATCC AGCAGCTTTT CGGAAAATGC    -1001

ATTTGCTGTT CGGAGTTTAA TCAGAAGACG ATTCCTGCCT CCGTCCCCGG
CTCCTTCATC GTCCCATCTC CCCTGTCTCT CTCCTGGGGA GGCGTGAAGC
GGTCCCGTGG ATAGAGATTC ATGCCTGTGT CCGCGCGTGT GTGCGCGCGT
ATAAATTGCC GAGAAGGGGA AAACATCACA GGACTTCTGC GAATACCGGA
CTGAAAATTG TAATTCATCT GCCGCCGCCG CTGCCAAAAA AAAACTCGAG
CTCTTGAGAT CTCCGGTTGG GATTCCTGCG GATTGACATT TCTGTGAAGC
AGAAGTCTGG GAATCGATCT GGAAATCCTC CTAATTTTTA CTCCCTCTCC
CCCCGACTCC TGATTCATTG GGAAGTTTCA AATCAGCTAT AACTGGAGAG
TGCTGAAGAT TGATGGGATC GTTGCCTTAT GCATTTGTTT TGGTTTTACA
AAAAGGAAAC TTGACAGAGG ATCATGCTGT ACTTAAAAAA TACAAGTAAG    -501

TCTCGCACAG GAAATTGGTT TAATGTAACT TTCAATGGAA ACCTTTGAGA
TTTTTTACTT AAAGTGCATT CGAGTAAATT TAATTTCCAG GCAGCTTAAT
ACATTGTTTT TAGCCGTGTT ACTTGTAGTG TGTATGCCCT GCTTTCACTC
AGTGTGTACA GGGAAACGCA CCTGATTTTT TACTTATTAG TTTGTTTTTT
CTTTAACCTT TCAGCATCAC AGAGGAAGTA GACTGATATT AACAATACTT
ACTAATAATA ACGTGCCTCA TGAAATAAAG ATCCGAAAGG AATTGGAATA
AAAATTTCCT GCGTCTCATG CCAAGAGGGA AACACCAGAA TCAAGTGTTC
CGCGTGATTG AAGACACCCC CTCGTCCAAG AATGCAAAGC ACATCCAATA
AAATAGCTGG ATTATAACTC CTCTTCTTTC TCTGGGGGCC GTGGGGTGGG
AGCTGGGGCG AGAGGTGCCG TTGGCCCCCG TTGCTTTTCC TCTGGGAAGG    -1
```

FIG. 2A

1	MET	ALA	HIS	ALA	GLY	ARG	THR	GLY	TYR	ASP	ASN	ARG	GLU	ILE	VAL
	ATG	GCG	CAC	GCT	GCG	AGA	ACG	GGG	TAC	GAC	AAC	CGG	GAG	ATA	GTG
	MET	LYS	TYR	ILE	HIS	TYR	LYS	LEU	SER	GLN	ARG	GLY	TYR	GLU	TRP
46	ATG	AAG	TAC	ATC	CAT	TAT	AAG	CTG	TCG	CAG	AGG	GGC	TAC	GAG	TGG
	ASP	ALA	GLY	ASP	VAL	GLY	ALA	ALA	PRO	PRO	GLY	ALA	ALA	PRO	ALA
91	GAT	GCG	GGA	GAT	GTG	GGC	GCC	GCG	CCC	CCG	GGG	GCC	GCC	CCC	GCA
	PRO	GLY	ILE	PHE	SER	SER	GLN	PRO	GLY	HIS	THR	PRO	HIS	PRO	ALA
136	CCG	GGC	ATC	TTC	TCC	TCC	CAG	CCC	GGG	CAC	ACG	CCC	CAT	CCA	GCC
	ALA	SER	ARG	ASP	PRO	VAL	ALA	ARG	THR	SER	PRO	LEU	GLN	THR	PRO
181	GCA	TCC	CGC	GAC	CCG	GTC	GCC	AGG	ACG	TCG	CCG	CTG	CAG	ACC	CCG
	ALA	ALA	PRO	GLY	ALA	ALA	ALA	GLY	PRO	ALA	LEU	SER	PRO	VAL	PRO
226	GCT	GCC	CCC	GGC	GCC	GCC	GCG	GGG	CCT	GCG	CTC	AGC	CCG	GTG	CCA
	PRO	VAL	VAL	HIS	LEU	ALA	LEU	ARG	GLN	ALA	GLY	ASP	ASP	PHE	SER
271	CCT	GTG	GTC	CAC	CTG	GCC	CTC	CGC	CAA	GCC	GGC	GAC	GAC	TTC	TCC
	ARG	ARG	TYR	ARG	GLY	ASP	PHE	ALA	GLU	MET	SER	SER	GLN	LEU	HIS
316	CGC	CGC	TAC	CGC	GGC	GAC	TTC	GCC	GAG	ATG	TCC	AGC	CAG	CTG	CAC
	LEU	THR	PRO	PHE	THR	ALA	ARG	GLY	ARG	PHE	ALA	THR	VAL	VAL	GLU
361	CTG	ACG	CCC	TTC	ACC	GCG	CGG	GGA	CGC	TTT	GCC	ACG	GTG	GTG	GAG
	GLU	LEU	PHE	ARG	ASP	GLY	VAL	ASN	TRP	GLY	ARG	ILE	VAL	ALA	PHE
406	GAG	CTC	TTC	AGG	GAC	GGG	GTG	AAC	TGG	GGG	AGG	ATT	GTG	GCC	TTC
	PHE	GLU	PHE	GLY	GLY	VAL	MET	CYS	VAL	GLU	SER	VAL	ASN	ARG	GLU
451	TTT	GAG	TTC	GGT	GGG	GTC	ATG	TGT	GTG	GAG	AGC	GTC	AAC	CGG	GAG
	MET	SER	PRO	LEU	VAL	ASP	ASN	ILE	ALA	LEU	TRP	MET	THR	GLU	TYR
496	ATG	TCC	CCC	CTG	GTG	GAC	AAC	ATC	GCC	CTG	TGG	ATG	ACT	GAG	TAC
	LEU	ASN	ARG	HIS	LEU	HIS	THR	TRP	ILE	GLN	ASP	ASN	GLY	GLY	TRP
541	CTG	AAC	CGG	CAC	CTG	CAC	ACC	TGG	ATC	CAG	GAT	AAC	GGA	GGC	TGG
	ASP	ALA	PHE	VAL	GLU	LEU	TYR	GLY	PRO	SER	MET	ARG	PRO	LEU	PHE
586	GAT	GCC	TTT	GTG	GAA	CTG	TAC	GGC	CCC	AGC	ATG	CGG	CCT	CTG	TTT
	ASP	PHE	SER	TRP	LEU	SER	LEU	LYS	THR	LEU	LEU	SER	LEU	ALA	LEU
631	GAT	TTC	TCC	TGG	CTG	TCT	CTG	AAG	ACT	CTG	CTC	AGT	TTG	GCC	CTG
	VAL	GLY	ALA	CYS	ILE	THR	LEU	GLY	ALA	TYR	LEU	SER	HIS	LYS	
676	GTG	GGA	GCT	TGC	ATC	ACC	CTG	GGT	GCC	TAT	CTG	AGC	CAC	AAG	TGA

FIG. 2B

```
        AGTCAACATC CCTGCCCCAA ACAAATATGC
        AAAAGCTTCA CTAAAGCACT AGAAATAATA TGCATTGTCA GTGATGTACC
        ATGAAACAAA GCTGCAGGCT GTTTAAGAAA AAATAACACA CATATAAACA
        TCACACACAC AGACAGACAC ACACACACAC AACAATTAAC AGTCTTCAGG
        CAAAACGTCG AATCAGCTAT TTACTGCCAA AGGGAAATAT CATTTATTTT
        TTACATTATT AAGAAAAAAG ATTTATTTAT TTAAGACAGT CCCATCAAAA

1001    CTCCGTCTTT GGAAATCCGA CCACTAATTG CCAAACACCG CTTCGTGTGG
        CTCCACCTGG ATGTTCTGTG CCTGTAAACA TAGATTCGCT TTCCATGTTG
        TTGGCCGGAT CACCATCTGA AGAGCAGACG GATGGAAAAA GGACCTGATC
        ATTGGGGAAG CTGGCTTTCT GGCTGCTGGA GGCTGGGGAG AAGGTGTTCA
        TTCACTTGCA TTTCTTTGCC CTGGGGGCGT GATATTAACA GAGGGAGGGT
        TCCCGTGGGG GGAAGTCCAT GCCTCCCTGG CCTGAAGAAG AGACTCTTTG
        CATATGACTC ACATGATGCA TACCTGGTGG GAGGAAAAGA GTTGGGAACT
        TCAGATGGAC CTAGTACCCA CTGAGATTTC CACGCCGAAG GACAGCGATG
        GGAAAAATGC CCTTAAATCA TAGGAAAGTA TTTTTTTAAG CTACCAATTG
        TGCCGAGAAA AGCATTTTAG CAATTTATAC AATATCATCC AGTACCTTAA

1501    ACCCTGATTG TGTATATTCA TATATTTTGG ATACGCACCC CCCAACTCCC
        AATACTGGCT CTGTCTGAGT AAGAAACAGA ATCCTCTGGA ACTTGAGGAA
        GTGAACATTT CGGTGACTTC CGATCAGGAA GGCTAGAGTT ACCCAGAGCA
        TCAGGCCGCC ACAAGTGCCT GCTTTTAGGA GACCGAAGTC CGCAGAACCT
        ACCTGTGTCC CAGCTTGGAG GCCTGGTCCT GGAACTGAGC CGGGCCCTCA
        CTGGCCTCCT CCAGGGATGA TCAACAGGGT AGTCTGGTCT CCGAATGTCT
        GGAAGCTGAT GGATGGAGCT CAGAATTCCA CTGTCAAGAA AGAGCAGTAG
        AGGGGTGTGG CTGGGCCTGT CACCCTGGGG CCCTCCAGGT AGGCCCCGTTT
        TCACGTGGAG CATAGGAGCC ACGACCCTTC TTAAGACATG TATCACTGTA
        GAGGGAAGGA ACACAGGCCC TGGGCCTTCC TATCAGAAGG ACATGGTGAA

2001    GGCTGGGAAC GTGAGGAGAG GCAATGGCCA CGGCCCATTT TGGCTGTAGC
        ACATGGCACG TTGGCTGTGT GGCCTTGGCC ACCTGTGAGT TTAAAGCAAG
        GCTTTAAATG ACTTTGGAGA GGGTCACAAA TCCTAAAAGA AGCATTGAAC
        TGAGGTGTCA TGGATTAATT GACCCCTGTC TATGGAATTA CATGTAAAAC
        ATTATCTTGT CACTGTAGTT TGGTTTTATT TGAAAACCTG ACAAAAAAAA
        AGTTCCAGGT GTGGAATATG GGGGTTATCT GTACATCCTG GGGCATTAAA
        AAAAAATCAA TGGTGGGGAA CTATAAAGAA GTAACAAAAG AAGTGACATC
        TTCAGCAAAT AAACTAGGAA ATTTTTTTTT CTTCCAGTTT AGAATCAGCC
        TTGAAACATT GATGGAATAA CTCTGTGGCA TTATTGCATT ATATACCATT
        TATCTGTATT AACTTTGGAA TGTACTCTGT TCAATGTTTA ATGCTGTGGT
```

FIG. 2C

2501 TGATATTTCG AAAGCTGCTT TAAAAAAATA CATGCATCTC AGCGTTTTTT
TGTTTTTAAT TGTATTTAGT TATGGCCTAT ACACTATTTG TGAGCAAAGG
TGATCGTTTT CTGTTTGAGA TTTTTATCTC TTGATTCTTC AAAAGCATTC
TGAGAAGGTG AGATAAGCCC TGAGTCTCAG CTACCTAAGA AAAACCTGGA
TGTCACTGGC CACTGAGGAG CTTTGTTTCA ACCAAGTCAT GTGCATTTCC
ACGTCAACAG AATTGTTTAT TGTGACAGTT ATATCTGTTG TCCCTTTGAC
CTTGTTTCTT GAAGGTTTCC TCGTCCCTGG GCAATTCCGC ATTTAATTCA
TGGTATTCAG GATTACATGC ATGTTTGGTT AAACCCATGA GATTCATTCA
GTTAAAAATC CAGATGGCGA ATGACCAGCA GATTCAAATC TATGGTGGTT
TGACCTTTAG AGAGTTGCTT TACGTGGCCT GTTTCAACAC AGACCCACCC

3001 AGAGCCCTCC TGCCCTCCTT CCGCGGGGGC TTTCTCATGG CTGTCCTTCA
GCGTCTTCCT GAAATGCAGT GGTCGTTACG CTCCACCAAG AAAGCAGGAA
ACCTGTGGTA TGAAGCCAGA CCTCCCCGGC GGGCCTCAGG GAACAGAATG
ATCAGACCTT TGAATGATTC TAATTTTTAA GCAAAATATT ATTTTATGAA
AGGTTTACAT TGTCAAAGTG ATGAATATGG AATATCCAAT CCTGTGCTGC
TATCCTGCCA AAATCATTTT AATGGAGTCA GTTTGCAGTA TGCTCCACGT
GGTAAGATCC TCCAAGCTGC TTTAGAAGTA ACAATGAAGA ACGTGGACGT
TTTTAATATA AAGCCTGTTT TGTCTTTTGT TGTTGTTCAA ACGGGATTCA
CAGAGTATTT GAAAAATGTA TATATATTAA GAGGTCACGG GGGCTAATTG
CTAGCTGGCT GCCTTTTGCT GTGGGGTTTT GTTACCTGGT TTTAATAACA

3501 GTAAATGTGC CCAGCCTCTT GGCCCCAGAA CTGTACAGTA TTGTGGCTGC
ACTTGCTCTA AGAGTAGTTG ATGTTGCATT TTCCTTATTG TTAAAAACAT
GTTAGAAGCA ATGAATGTAT ATAAAAGCAA AAAAAAAAAA AAAAAAA

FIG. 2D

TGATTGAAGACACCCCCTCGTCCAAGAATGCAAAGCACATCCAATAAAATAGCTGGATTATAACTCCTCTTCTTTCTCTG

+1

GGGGCCGTGGGGTGGGAGCTGGGGCCGAGAGGTGCCGTTGGCCCCCGTTGCTTTTCCTCTGGGAAGG MET ALA HIS
ATG GCG CAC

ALA GLY ARG THR GLY TYR ASP ASN ARG GLU ILE VAL MET LYS TYR ILE HIS TYR LYS LEU
GCT GGG AGA ACG GGG TAC GAC AAC CGG GAG ATA GTG ATG AAG TAC ATC CAT TAT AAG CTG

100

SER GLN ARG GLY TYR GLU TRP ASP ALA GLY ASP VAL GLY ALA ALA PRO PRO GLY ALA ALA
TCG CAG AGG GGC TAC GAG TGG GAT GCG GGA GAT GTG GGC GCC GCG CCC CCG GGG GCC GCC

PRO ALA PRO GLY ILE PHE SER SER GLN PRO GLY HIS THR PRO HIS PRO ALA ALA SER ARG
CCC GCA CCG GGC ATC TTC TCC TCC CAG CCC GGG CAC ACG CCC CAT CCA GCC GCA TCC CGC

200

ASP PRO VAL ALA ARG THR SER PRO LEU GLN THR PRO ALA ALA PRO GLY ALA ALA ALA GLY
GAC CCG GTC GCC AGG ACC TCG CCG CTG CAG ACC CCG GCT GCC CCC GGC GCC GCC GCG GGG

300

PRO ALA LEU SER PRO VAL PRO PRO VAL VAL HIS LEU ALA LEU ARG GLN ALA GLY ASP ASP
CCT GCG CTC AGC CCG GTG CCA CCT GTG GTC CAC CTG GCC CTC CGC CAA GCC GGC GAC GAC

PHE SER ARG ARG TYR ARG GLY ASP PHE ALA GLU MET SER SER GLN LEU HIS LEU THR PRO
TTC TCC CGC CGC TAC CGC GGC GAC TTC GCC GAG ATG TCC AGC CAG CTG CAC CTG ACG CCC

400

PHE THR ALA ARG GLY ARG PHE ALA THR VAL VAL GLU GLU LEU PHE ARG ASP GLY VAL ASN
TTC ACC GCG CGG GGA CGC TTT GCC ACG GTG GTG GAG GAG CTC TTC AGG GAC GGG GTG AAC

TRP GLY ARG ILE VAL ALA PHE PHE GLU PHE GLY GLY VAL MET CYS VAL GLU SER VAL ASN
TGG GGG AGG ATT GTG GCC TTC TTT GAG TTC GGT GGG GTC ATG TGT GTG GAG AGC GTC AAC

500

ARG GLU MET SER PRO LEU VAL ASP ASN ILE ALA LEU TRP MET THR GLU TYR LEU ASN ARG
CGG GAG ATG TCG CCC CTG GTG GAC AAC ATC GCC CTG TGG ATG ACT GAG TAC CTG AAC CGG

600

HIS LEU HIS THR TRP ILE GLN ASP ASN GLY GLY TRP VAL GLY ALA SER GLY ASP VAL SER
CAC CTG CAC ACC TGC ATC CAG GAT AAC GGA GGC TGG GTA GGT GCA TCT GGT GAT GTG AGT

LEU GLY OPA GGCCACAGGTCCGAGATCGGGGGTTGGAGTGCGGGTGGGCTCCTGGGCAATGGGAGGCTGTGGAGCC
CTG GGC TGA

FIG. 3